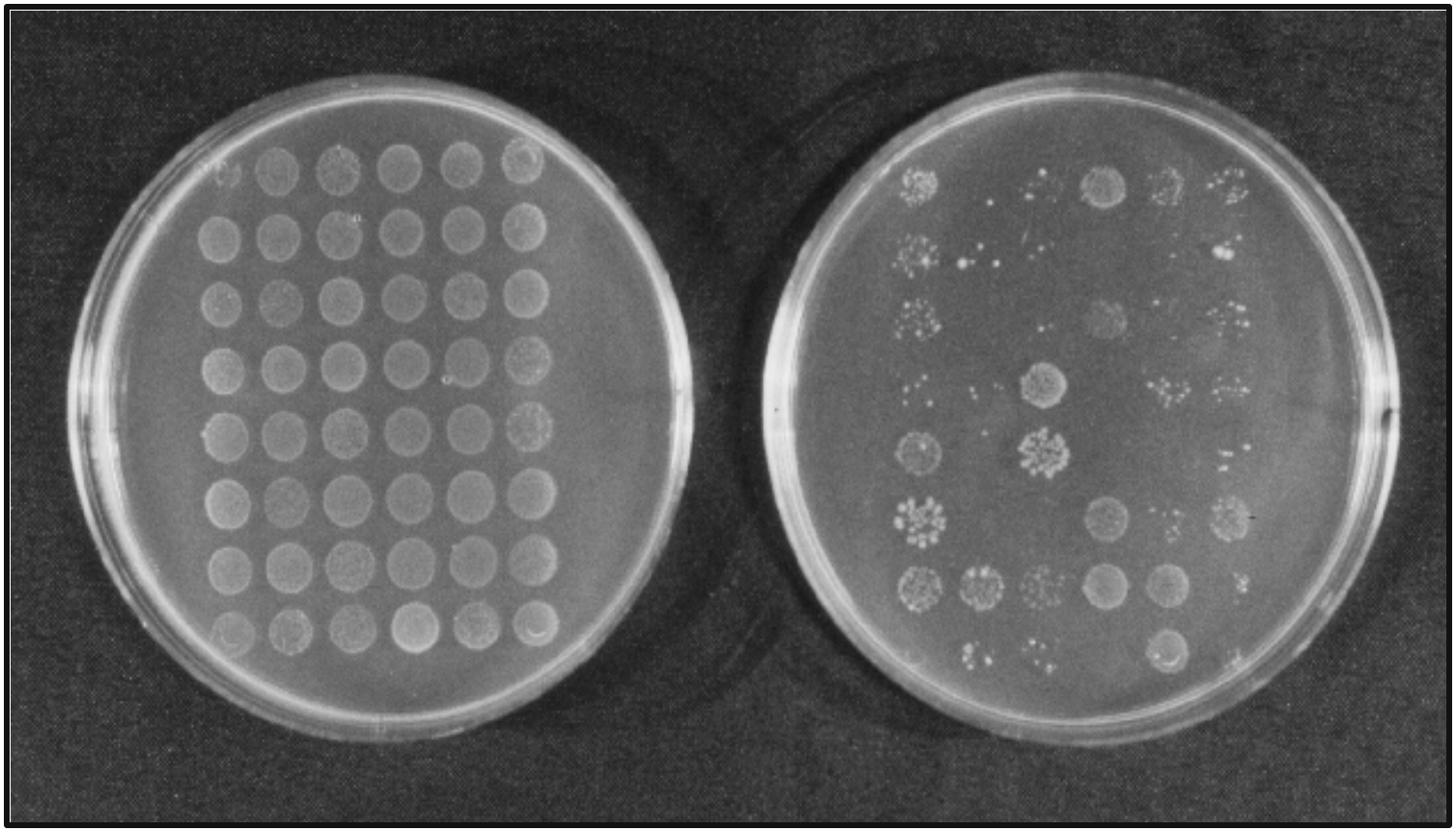
Microbial Source Tracking

- Phenotypic methods (biochemical based)
  - Antibiotic resistance analysis (ARA)
  - Carbon utilization profile (CUP)

* Both are library dependent methods

Antibiotic Resistance Analysis



Patterns of Antibiotic Resistance

Antibiotic (mg/l)	Poultry	Humans
	----% of isolate resistant----	
Erythromycin (15)	77	76
Neomycin (40)	98	4
Tetracycline (100)	98	12

Adapted from Hagedorn et al. (1999)

Antibiotic Resistance Analysis (ARA)

Advantages

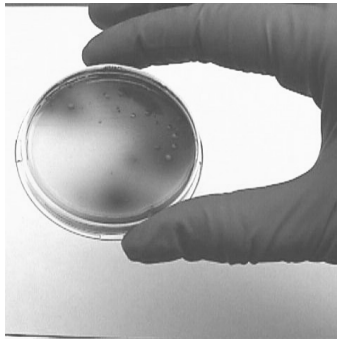
- Easy to type
- Easy to perform
- Easy to interpret
- Inexpensive

Disadvantages

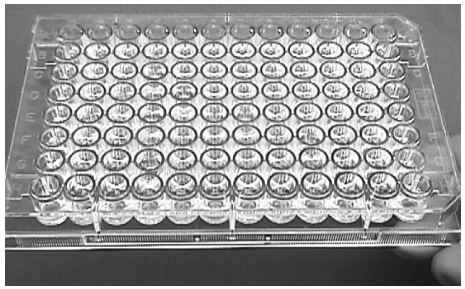
- Antibiotic resistance can be transferred
- Antibiotic dependent
- Culture dependent

Carbon utilization (91 isolates + 5 controls)

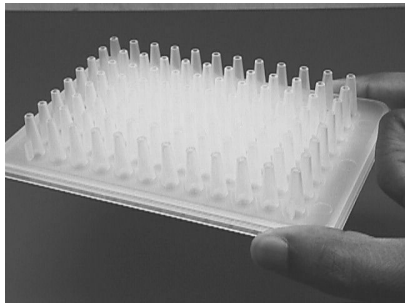
Enterococcosel agar



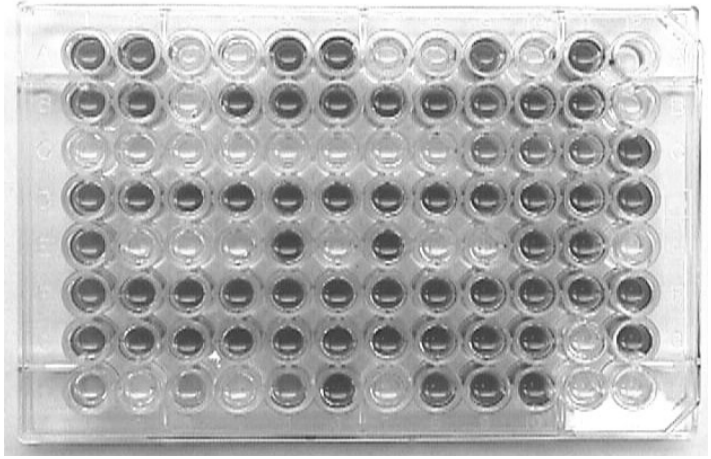
150 mL phosphate buffer



Replicator



1) BHI with 6.5% NaCl
2) arginine hydrolysis
3) pyruvate utilization
4) arabinose utilization
5) L-sorbose utilization
6) raffinose utilization
+ catalase and cell morph.



Carbon Utilization Profile

Advantages

- Easy to type
- Easy to perform
- Easy to interpret
- Relatively inexpensive
- Automated analysis

Disadvantages

- Unstable markers
- Ecologically irrelevant
- Culture dependent

Why molecular techniques?

- Genotyping methods are more sensitive and stable
- Do not rely on the culturability of microorganisms
- Simultaneous detection/monitoring of different groups without the requirement of a library

Steps in Fingerprinting Methods

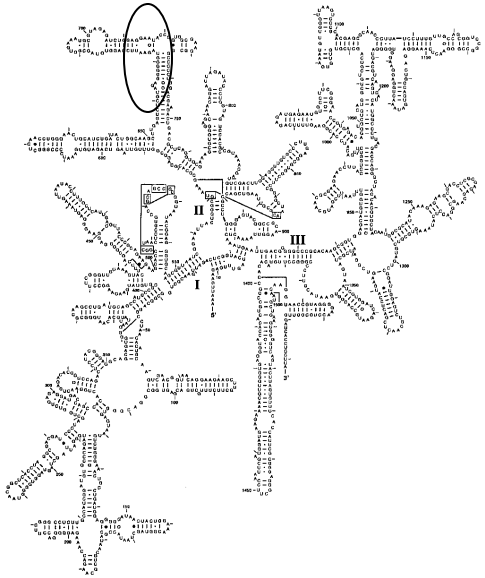
- Bacterial isolation
- ID confirmation
- DNA extractions
- PCR-amplification or DNA digestion
- Gel electrophoresis (and Southern hybridization)
- Image capture and analysis
- Average Rate of Correct Classification

DNA Fingerprinting Methods

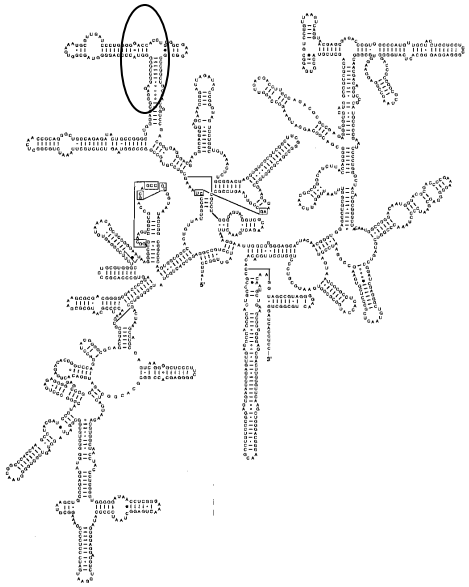
- RFLP analysis
- RAPD
- rep-PCR
- Sequencing ITS regions
- PCR-amplification of specific insertion sequences (IS)
- PFGE (gold standard in outbreak research)
- AFLP

Comparative Sequence Analysis of 16S rRNA

16S rRNA of
Escherichia coli



16S rRNA of
Acinetobacter calcoaceticus



	650	660	670
E. coli	**GCUUGAGUCU**	**CGUAGAGGGG**	**GGUAGAAUUC**
A. calcoaceticus	**GCUAGAGUAU**	**GGGAGAGGAU**	**GGUAGAAUUC**

MST Molecular Methods

Genotypic Methods – library dependent

-- PFGE

-- Ribotyping

-- rep-PCR

-- AFLP

MST Molecular Methods

- **Library independent methods**
  - **T-RFLP**
  - **LH-PCR**